

BEACON ANALYTICAL SYSTEMS
DON (G) PLATE KIT

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GENERAL INFORMATION

DON (G) Plate Kit is a competitive ELISA for the quantitative analysis of deoxynivalenol (DON) in corn and wheat.

The instructions presented in this document cover only the procedure for performing the analytical test for official inspections. For questions regarding this procedure, contact Dr. Ajit Ghosh of the Technology and Science Division by phone at 816-891-0417 or email at Ajit.K.Ghosh@usda.gov.

Refer to the current policies and/or instructions issued by the Policies, Procedures, and Market Analysis Branch (PPMAB) of the Field Management Division for information on use of this test kit in official inspections including sampling, general sample preparation (e.g., grinding and dividing), reporting and certification of test results, laboratory safety, and hazardous waste management. For questions regarding these policies and/or instructions, contact Patrick McCluskey of PPMAB by phone at 816-659-8403 or email at Patrick.J.McCluskey@usda.gov.

Approved Test Kit Information

Test Kit Vendor:	Beacon Analytical Systems Inc. 207-571-4302
Test Kit Name:	DON (G) Plate Kit
Product Number:	20-0256
Effective Date of Instruction:	3/24/2015
Instruction Revision Number:	0
Conformance Range:	0.5 – 5.0 ppm
Number of Analyses to Cover Conformance Range:	1
Type of Service:	Quantitative
Supplemental Analysis:	No
Approved Commodities:	Corn and wheat
Extraction Method:	Shake 50 gram ground sample with 250 milliliters (mL) of distilled or deionized water for 3 minutes.
Test Format:	Microtiter well plate assay
Detection Method:	Stat Fax Reader, Model 303 Plus

PREPARATION OF TESTING MATERIALS AND EQUIPMENT

Wash Solution and reagents:

Mix the contents of one (1) wash solution packet with 1 liter of distilled or deionized water. Fill a wash bottle with wash solution.

Allow kit reagents (DON-HRP conjugate, calibrators, antibody, substrate, stop solution and plate) to reach room temperature prior to running the test.

Stat Fax 303 Plus reader calibration:

1. Turn the power switch on.
2. Look for **Ready** on the screen.
3. Press **ABS**.
4. Press **2** (450 nm) and **Enter**.
5. Press **4** (630 nm) and **Enter**.
6. Place the empty carrier on the track with alignment.
7. Press **Enter** and see the values (Max \pm 0.005).
8. Press **Clear** twice to quit.
9. Perform this procedure every time you turn the reader on.

Set up for an initial test (this is one time procedure):

1. Press **ALT**.
2. Press **YES** when STRIP TYPE Y/N displayed.
3. Press **YES** when 8 wells Y/N displayed.
4. The display shows Ready.
5. Press **MULT**.
6. Press **YES** when Regression Y/N displayed.
7. The display shows SELECT FILTER.
8. Press **2** for 450 nm and **Enter**.

9. The display shows **SELECT DIF FILTER**.
10. Press **4** for 630 nm and **Enter**.
11. Press **NO** when Linear Y/N displayed.
12. Keep selecting **NO** until Log/Logit Y/N displayed.
13. Press **YES** when Log/Logit Y/N displayed.
14. Press **NO** when BLANK Y/N displayed.
15. Press **NO** when Duplicates Y/N displayed.
16. Press **5** and **Enter** when # of Cals displayed.
17. Press **YES** when Select Units Y/N displayed.
18. Press **11** and **Enter** when Key unit code # displayed.
19. Press **YES** when ppm Y/N displayed.
20. Press **0.4** and **Enter** when Cal2 displayed.
21. Press **1** and **Enter** when Cal3 displayed.
22. Press **2.5** and **Enter** when Cal4 displayed.
23. Press **6** and **Enter** when Cal5 displayed.
24. Press **NO** when Off Curve Ok Y/N displayed.
25. The display shows Set Carrier to 1.
26. Put a strip with calibrators (and samples) in the carrier (right position). Press **Enter**.
(0 ppb of calibrator is read first)
27. Wait until lamp warmed up.
28. Press **NO** when PLOT Curve Y/N displayed.
29. Press **YES** when Accept Curve Y/N displayed. (Only if the R^2 value is higher than 0.990)
30. The display shows Set Carrier.
31. Press **ALT**.
32. Press **YES** when SAVE TEST Y/N displayed.
33. Press **YES** when NAME TEST Y/N displayed.

34. Select character by pressing 4 or 6. Press **Enter**.
35. Repeat this step to complete the TEST name.
(Press **Enter** a second time immediately after the last character was chosen)
36. The TEST will be saved as TEST # 2 if there is a saved TEST as #1.

EXTRACTION PROCEDURES

1. Weigh 50 ± 0.2 grams ground sample in a Whirl-pak stand-up bag.
2. Add 250 mL of distilled or deionized water.
3. Spin the bag to fold and twist the tabs to close tightly.
4. Vigorously shake for 3 minutes by hand.
5. Transfer 1 mL of sample extract into a micro-centrifuge tube.
6. Centrifuge for 3 min at 12,000 rpm.
7. Mix 0.1 mL of extract with 0.9 mL of distilled or deionized water.
8. Run the assay within 1 hr in order to keep the integrity of the extract.
9. Proceed to TEST PROCEDURES.

TEST PROCEDURES

1. Place the appropriate number of test wells into the microwell holder for all Calibrators and samples to be tested.
2. Mix each reagent by swirling the reagent bottle prior to use.

Well Location	1	2	3	4	5	6	7	8
Calibrators and Samples	C 0 ppm	C 0.4 ppm	C 1.0 ppm	C 2.5 ppm	C 6.0 ppm	Test Sample	Test Sample	Test Sample

3. Place 50 μ L of DON-HRP conjugate to each test well.
4. Using a new pipette tip for each, transfer 50 μ L of each calibrator (0, 0.4, 1.0, 2.5, and 6.0 ppm) and samples to the designated wells.

5. Using a multi-channel pipettor, transfer 50 µL of the antibody to each wells.
6. Mix by sliding the microwell holder back and forth on a flat surface for 10-20 seconds without splashing the reagents from the wells. Incubate for 10 minutes at room temperature.
7. Discard the contents of the wells. Fill the wells with wash solution and dump them out. Repeat this step 5 times, then turn the wells upside-down and tap out on a paper towel until the remaining wash solution has been removed.
8. Pour the needed volume of substrate into the reagent boat.
9. With new tips on the multi-channel pipettor, prime and pipette 100 µL of substrate into the wells and mix by sliding back and forth on a flat surface for 10-15 seconds. Incubate for 5 minutes.
10. With new tips on the multi-channel pipettor, prime and pipette 100 µL of stop solution into the wells and mix by sliding back and forth on a flat surface for 10-15 seconds.
11. Wipe the bottom of the microwells with a lint free dry Kim wipe and read on the Stat Fax reader using a 450 nm. Air bubbles should be eliminated, as they could affect analytical results.

Reading the test results:

1. Press **Menu**.
2. Press **2** and **Enter** when Select Test displayed.
3. Press **NO** when Plot Curve Y/N displayed.
4. Press **NO** when Stored Curve displayed.
5. The display shows Set Carrier to 1.
6. Put a strip with Calibrators and samples in the carrier (right position).
(0 ppb of Calibrator is read first)
7. Press **Enter**.
8. Press **NO** when Plot Curve Y/N displayed.
9. Press **YES** when Accept Y/N displayed.
(Only if the R² value is higher than 0.990)
10. Concentrations of the samples will be calculated and printed on the paper.
11. Put a second strip with samples to read in the middle line.
(Move the carrier to right)
12. Press **Enter**.

13. Concentrations of the samples will be calculated and printed on the paper.
14. If there are more samples to read, repeat the procedures (step 11 and 12) with a new strip or Press **Clear** twice to quit.

REPORTING AND CERTIFYING TEST RESULTS

Refer to the current instructions issued by the Policies, Procedures, and Market Analysis Branch of the Field Management Division for reporting and certification of test results. For questions regarding these instructions, contact Patrick McCluskey (816-659-8403 or Patrick.J.McCluskey@udsa.gov).

EQUIPMENT AND SUPPLIES

1. Material provided in DON (G) Plate Kit.

The test kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8° C (1 year from date of manufacture).

- a. 1 Plate containing 12 test strips of 8 wells coated with antispecies antibodies, in an aluminized pouch with indicating desiccant.
- b. 5 vials, each containing 2 mL of DON Calibrators corresponding to 0, 0.4, 1.0, 2.5, and 6.0 µg/mL (ppm) DON.
- c. 1 vial containing 8 mL of DON-HRP Conjugate.
- d. 1 vial containing 8 mL of DON Antibody Solution.
- e. 1 vial containing 14 mL of Substrate.
- f. 1 vial containing 14 mL of Stop Solution. (Caution! 1N HCl. Handle with care.)
- g. 1 packet Wash Solution.
- h. 1 Instructional Booklet.

2. Materials Required but not Provided

- a. Distilled or deionized water.
- b. Whirl-pak stand-up bags or equivalent.
- c. Micro-centrifuge and tube.
- d. Stat-Fax Reader Model 303 Plus equipped with a 450-nm filter (Awareness Technology Inc.).
- e. Pipettes and disposable tips capable of dispensing 50, 100, 900 and 1000 µL.

- f. Multi-channel (8-channel) or Eppendorf Repeater pipette, capable of dispensing 50 μ L, and 100 μ L.
- g. Vortex mixer.
- h. Wash bottle.
- i. Timer

STORAGE CONDITIONS AND PRECAUTIONS

1. Storage Conditions

- a. The reagents supplied with the test kit can be used until the expiration date on the kit label when stored refrigerated at temperatures between 2 -8° C. **(DO NOT FREEZE)**
- b. Return any unused microwells to their original foil bag and reseal them together with the desiccant provided.
- c. The substrate solution is light sensitive, therefore, avoid exposure to direct light.

2. Precautions

- a. Each reagent is optimized for use in DON (G) Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon DON (G) Plate Kits with different Lot numbers.
- b. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- c. Do not use reagents after expiration date.
- d. Reagents should be brought to room temperature, 20 – 28° C (62 – 82°F) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
- e. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. If contact should occur, immediately flush with copious amounts of water. Immediately clean up any spills and wash area with copious amounts of water.
- f. The use of a multichannel pipette to dispense the generic reagents (DON-HRP Conjugate, Antibody, Substrate and Stop Solution) is recommended when running 2 strips or more.
- g. The intended user of this kit is a trained laboratory technician. Familiarity with ELISA is recommended. Please contact Beacon Analytical Systems Inc. (www.beaconkits.com) for technical support if you have any questions about the use of this kit.

REVISION HISTORY

Revision 0 (3/24/2015)